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The enhancement of aminonucleoside nephrosis by the co-administration of protamine

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The enhancement of aminonucleoside nephrosis by the co-administration of protamine. An experimental model of focal segmental glomerular sclerosis (FSGS) was developed in rats by the combined administration of puromycin–aminonucleoside (AMNS) and protamine sulfate (PS). Male Sprague–Dawley rats, uninephrectomized three weeks before, received daily injections of subcutaneous AMNS (1 mg/100 g body wt) and intravenous PS (2 separated doses of 2.5 mg/100 g body wt) for four days. The series of injections were repeated another three times at 10 day intervals. The animals were sacrificed on days 24, 52, and 80. They developed nephrotic syndrome and finally renal failure. The time–course curve of creatinine clearance dropped and showed significant difference ($P < 0.01$) from that of each control group, such as, AMNS alone, PS alone or saline injected. Their glomeruli showed changes of progressive FSGS. The ultrastructural studies in the initial stage revealed significant lack of particles of perfused ruthenium red on the lamina rara externa and marked changes in epithelial cell cytoplasm. Therefore, it is suggested that the administration of PS enhances the toxicity of AMNS on the glomerulus and readily produces progressive FSGS in rats resulting in the end–stage renal disease.

Focal segmental glomerular sclerosis (FSGS) is a condition in which some glomeruli develop segmental areas of sclerosis. Patients with this condition present with a nephrotic syndrome which is resistant to steroid therapy. There is frequent progression to end–stage renal disease and recurrence in the transplanted kidney.

There have been several attempts to develop experimental models of FSGS in order to study its pathogenesis and pathology. Michael and co-workers [1, 2] induced FSGS in uninephrectomized rats by the administration of aminonucleoside of puromycin (AMNS). The lesions induced in the rat glomeruli were comparable to that of FSGS in man. However, severe histological changes, including extensive interstitial fibrosis and renal dysfunction, were not a feature.

In order to produce a more severe FSGS lesion that progresses to end–stage renal disease and which more closely mimics the disease in humans, protamine sulfate (PS) was evaluated to determine whether it could potentiate the effect of AMNS. It has been known for some time that PS has a neutralizing effect on glomerular polyanion [3–5]. Recent studies suggest that PS also eliminates cationized immune complexes and thereby can ameliorate an immune complex nephritis [6–8]. In this paper we demonstrate that the effect of AMNS on the epithelial cells and glomerular polyanion is enhanced by the co-administration of PS, and produces progressive FSGS in uninephrectomized rats which results in end–stage renal disease and resembles closely human FSGS.

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Methods

Experimental design

The experimental design is depicted in Figure 1. Forty–two 10 to 12 week old, male Sprague–Dawley (SD) rats were used in this experiment. A left unilateral nephrectomy through a flank incision was performed on each rat under ether anesthesia. Three weeks after the nephrectomy, the rats were randomly divided into four specific groups (A through D). All the rats received daily injections for four days: Group A (15 rats) received AMNS and PS, Group B (11 rats) AMNS and saline, Group C (11 rats) PS alone and Group D (5 rats) saline alone. AMNS (Sigma Chemical Co., St. Louis, Missouri, USA) was administered subcutaneously in a dose of 1 mg per 100 g body weight as a 2% solution in 0.9% sodium chloride. PS (from salmon testicle, Grade X, Sigma Chemical Co.) was injected intravenously in two doses (separated by a 4 hour interval) of 2.5 mg per 100 g body weight as a 1% solution in 0.9% sodium chloride. The drug was administered in a divided dose to avoid the deleterious effect of the drug on the circulation. Saline (0.9% sodium chloride) was given intravenously in a dose of 0.5 ml per 100 g body weight.

All rats received further four–day series of injections at 10-day intervals. The dose of AMNS in these series of injections was reduced to 0.5 mg per 100 g body weight. Five rats in Group A and three rats in each of Group B and C were sacrificed on day 24 after the second series of injections and on day 52 after the fourth series, respectively. Five rats in each group receiving four series of injections were also sacrificed on day 80. In addition to the rats in the four groups above, a fifth group (Group E) of five non-nephrectomized rats was studied. Group E rats were subjected to the same protocol as Group A rats and were sacrificed on day 80.

All rats were maintained on standard rat diet pellets containing 20% of crude protein and 0.12% of sodium (Clark King & Co., Australia) and had free access to tap water throughout the course of experiment.

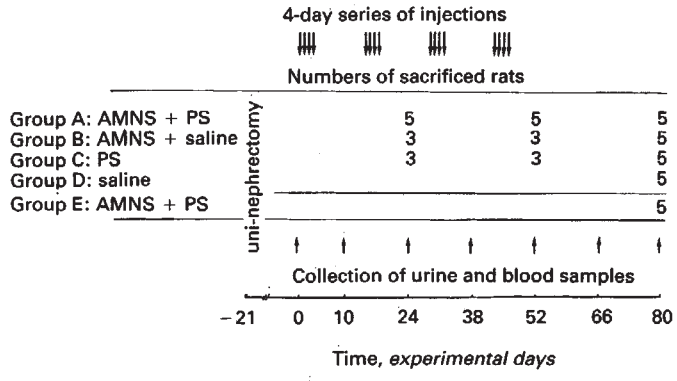

Fig. 1. *Experimental design.* Group A and E received aminonucleoside (AMNS) and Protamine sulfate (PS) in 4-day series of injections; Group B, aminonucleoside (AMNS) and saline; Group C, protamine sulfate (PS) alone; Group D, saline alone.

Laboratory studies

Twenty–four–hour urine samples were obtained from all rats (Groups A to E) in metabolic cages for an initial control period and then fortnightly during the course of the experiment. During collection periods the rats were allowed free access to water but no food. All urines were collected with thymol crystals and were kept at 4°C. Blood was obtained from the tail vein of each rat immediately following urine collection. Serum samples were separated by centrifuging at 3000 rpm for 15 minutes and stored at −20°C. Urinary protein was measured by the standard turbidimetric method with sulfosalicylic acid [9]. Determinations of urinary protein and creatinine and of serum albumin, total cholesterol, urea, and creatinine were carried out on Centrifichem 400 Centrifugal Analyzer (Union Carbide). Creatinine clearance was assessed from the results of urinary and serum creatinine and expressed as ml/min/kg body weight.

Data on the rats which were sacrificed on day 80 were statistically analyzed. The time–course curves were compared, using a two–way analysis of variance on each parameter (Figs. 2 and 3). Difference between groups or mean values was considered to be significant when $P < 0.05$.

Pathology

Light microscopy. Portions of kidney removed at nephrectomy and at sacrifice were fixed in 10% buffered formalin. Staining procedures were performed by standard techniques in our laboratory [10]. Paraffin sections were cut at 3 μm and stained with hematoxylin and eosin, periodic acid–Schiff (PAS) and Masson's trichrome. All glomeruli in randomly selected, high power fields were examined for evidence of focal sclerosis.

Histometrical measurements. One hundred and fifty to 200 glomeruli from each specimen were examined. The degree of sclerosis in each glomerulus was subjectively graded on a scale of 0 to 4: Grade 0, no change; Grade 1, sclerotic area less than or equal to ¼ of glomerulus or distinct adhesion present between capillary tuft and Bowman's capsule; Grade 2, sclerosis of ¼ to ½ total glomerular area; Grade 3, sclerosis of more than ½ the glomerulus but not global; Grade 4, global sclerosis. An Index of Sclerosis (I.S.) was calculated using the following formula:

$$\text{I.S.} = \frac{1 \times N_1 + 2 \times N_2 + 3 \times N_3 + 4 \times N_4}{N_0 + N_1 + N_2 + N_3 + N_4}$$

where N is the number of glomeruli in each grade of sclerosis.

The interstitial volume was estimated with the point counting technique modified from the method of Bennett, Walker and Kincaid–Smith [11]. Five portions of renal cortex in Masson's trichrome staining sections were randomly selected, and photographs were taken using Agfachrome 50L films with light microscopy photocamera (Leitz Dialux, magnification ×250). The micrographs were projected on a screen sized to 24 × 16cm where 121 (11 × 11) points were squarely plotted at a distance of 1.5cm, and hence 605 (121 × 5) points were analyzed in each case. The points of glomeruli and large vessels were excluded from total points. Relative interstitial volume was expressed as the percentage of points on interstitium to the total.

In these histometrical measurements, statistical analysis between groups or subgroups was performed using Student's t-test.

Immunoperoxdase technique. Portions of kidney were fixed in periodate–lysine–paraformaldehyde (PLP) for four hours, washed in phosphate buffered saline containing 7% sucrose several times and snap frozen. A indirect immunoperoxdase technique [12] was used for identifying immunoglobulins (Ig) and C3. Each 6 μm cryostat section was incubated with goat anti-rat IgG, goat anti-rat IgM, sheep anti-rat IgA and rabbit anti-rat C3 (Cappel, diluted 1:160) for 30 minutes, followed by peroxidase–labelled rabbit anti-goat Ig for IgG or IgM, peroxidase–labelled anti-sheep Ig for IgA and peroxidase–labelled swine anti-rabbit Ig for C3 (DAKO, diluted 1:80) for 15 minutes, respectively. Peroxidase activity was detected by using the substrate diaminobenzidine for 5 minutes. Sections were counterstained with hematoxylin.

Studies in the initial stage

Eighteen male, 10 to 12 week–old SD rats were randomly divided into three groups of six rats (Groups A′, B′, and C′). The animals in each group daily received injections according to the same protocol of group A, B, and C, respectively. Three rats were sacrificed on day 4 after three day–injections and another three on day 7 after 4 day–injections. The animals were kept in metabolic cages during the experiment. Twenty–four hour–urine was collected for assessment of urinary protein. At sacrifice, the animals were anesthetized with ether. The abdominal cavity was exposed by a middle line incision. Right kidney was removed after the ligature on right renal vessels and ureter, and prepared for light microscopy. Left kidney was perfused for 15 minutes with ruthenium red (RR) solution and then processed for electron microscopy by the method of Kanwar and Farquhar [13]. Perfusate was prepared as a 0.2% solution of RR in Karnovsky's aldehyde solution as described by Luft [14]. Thin sections were cut at 60 nm, mounted on carbon–coated grids, stained with uranyl acetate and lead citrate, and examined with JEOL 100C electron microscopy operating at 60kV (JEOL, Tokyo, Japan).

RR counts. Twenty areas of the GBM were randomly photographed and printed at a magnification of ×78,000. The total number of RR particles in the lamina rara externa (LRE) were counted in each photograph and the data expressed as the number of RR particles/μm of GBM.

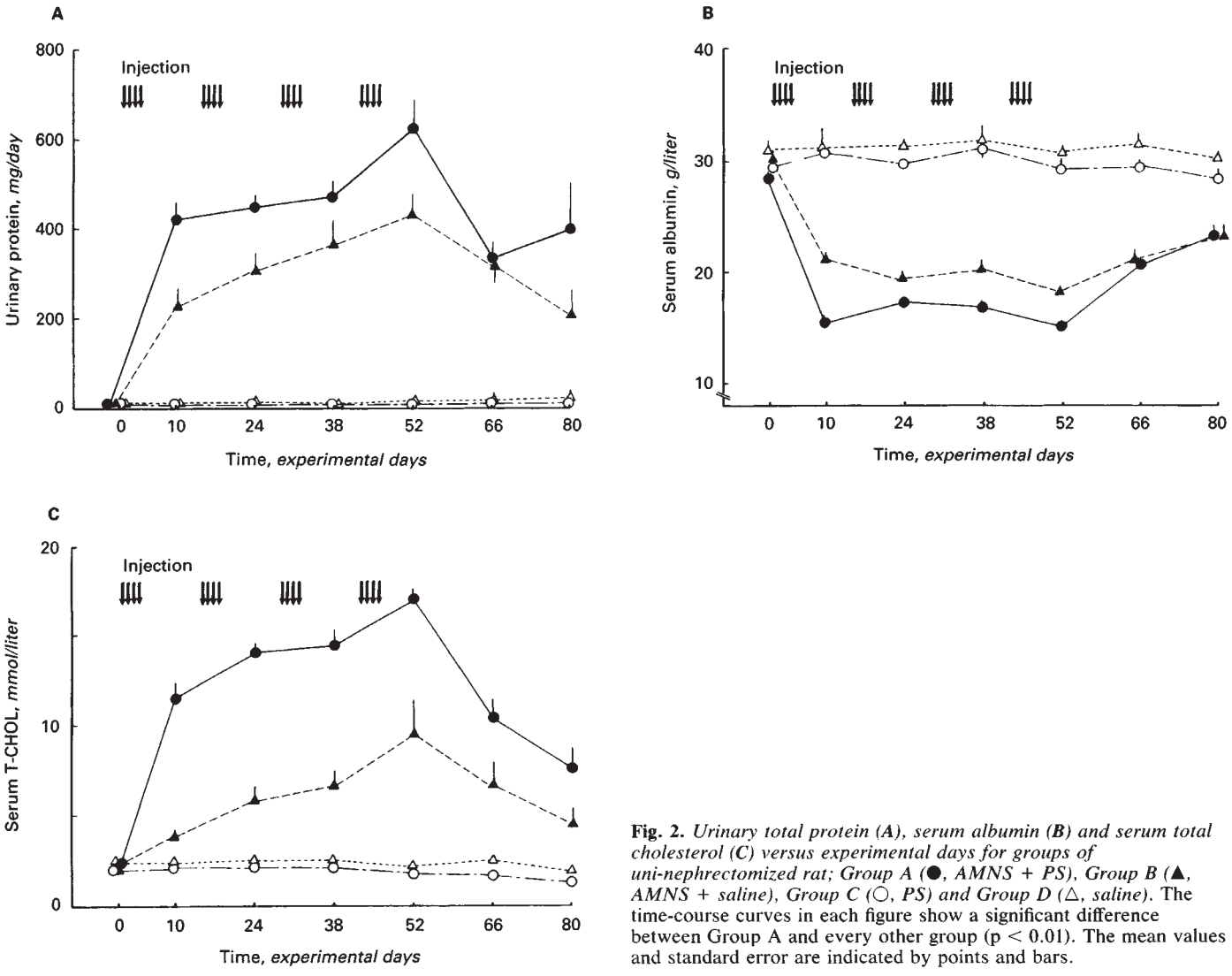

Fig. 2. *Urinary total protein (A), serum albumin (B) and serum total cholesterol (C) versus experimental days for groups of uni-nephrectomized rat; Group A (●, AMNS + PS), Group B (▲, AMNS + saline), Group C (○, PS) and Group D (△, saline).* The time-course curves in each figure show a significant difference between Group A and every other group ($p < 0.01$). The mean values and standard error are indicated by points and bars.

Another three non-treated SD rats were prepared for the RR-perfusion and compared with rats in Groups A′ through C′.

Results

There was a gradual increase in the mean body weight of each group of rats throughout the course of the experiment except in Group A rats in which there was a fall in mean body weight after day 52. Group A rats were anemic in the final stages of the experiment.

Biochemistry

All Group A rats showed marked proteinuria, hypoalbuminuria and hypercholesterolemia during the course of the experiment (Figs. 2 A–C). Similar but less marked, findings were present in Group B rats. Serum creatinine and urea levels were much higher in Group A rats than in rats of any other group (Figs. 3A and B). Renal failure was demonstrated by the decrease of creatinine clearance in group A rats (Fig. 3C). Rats in Groups C and D showed no abnormality in the biochemical parameters measured. The time–course curve of Group A in each parameter is significantly different from that of Group B, C, and D, respectively. Throughout the course of the experiment, rats in Group E had similar urinary protein, serum albumin and total cholesterol values as rats in Group A. There was, however, no rise in serum creatinine and urea and no decrease in creatinine clearance in non-nephrectomized rats. The relationship between non-nephrectomized and uninephrectomized groups in each parameter are shown in Table 1.

Pathology

Light microscopic findings in the chronic phase. Glomerular sclerotic lesions were characterized by adhesions to Bowman's capsule associated with collapse of capillaries and an increase in PAS-positive mesangial substance. There was slight swelling and vacuolization of visceral epithelial cells and mild epithelial proliferation. These findings were observed in glomeruli of the rats in Groups A, B, and E focally and segmentally. However, the changes on the same experimental day were much more severe in rats of Group A than in rats of Group B or E. The sclerosis in Group A rats which was already seen but mild on

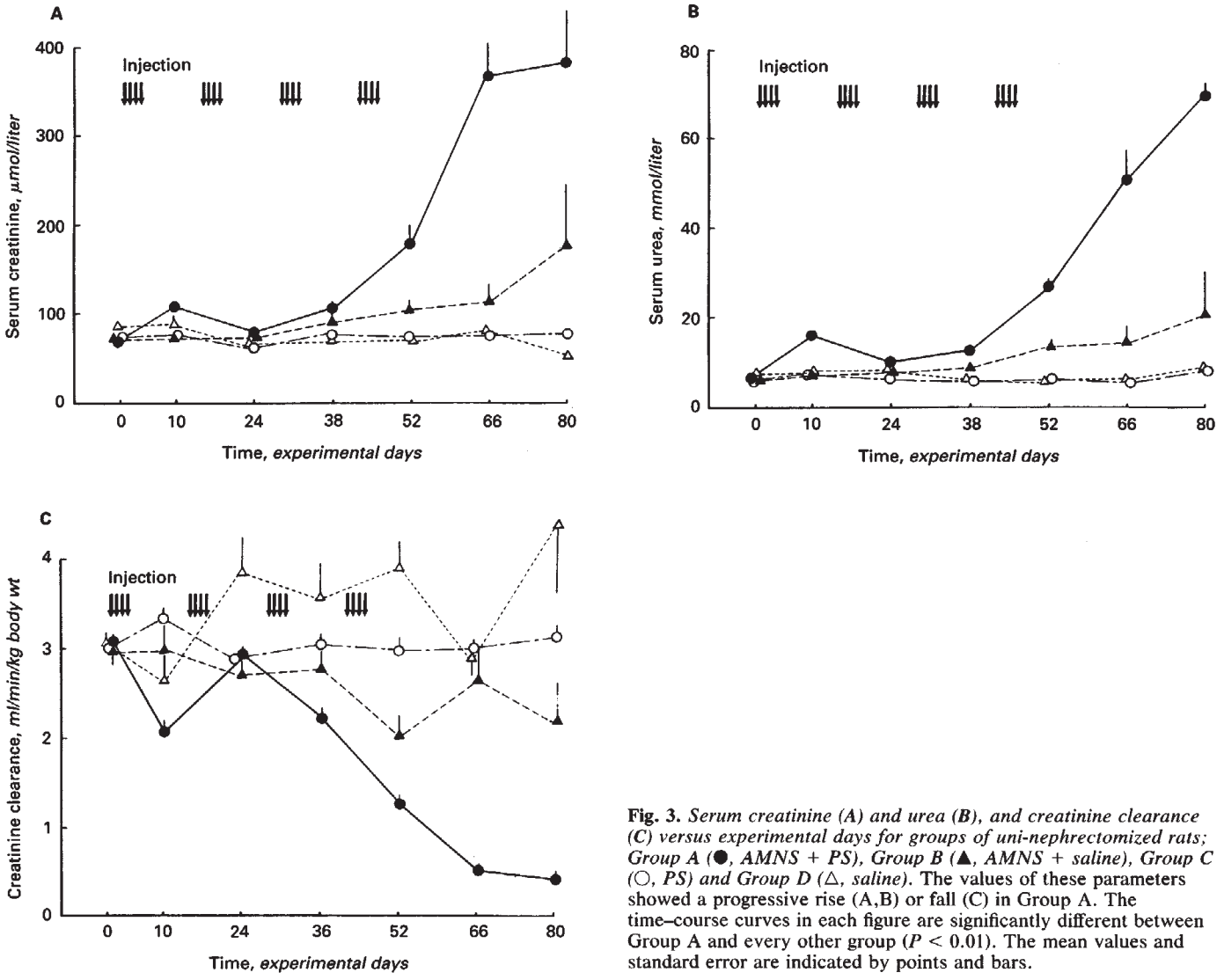

Fig. 3. *Serum creatinine (A) and urea (B), and creatinine clearance (C) versus experimental days for groups of uni-nephrectomized rats; Group A (●, AMNS + PS), Group B (▲, AMNS + saline), Group C (○, PS) and Group D (△, saline).* The values of these parameters showed a progressive rise (A,B) or fall (C) in Group A. The time–course curves in each figure are significantly different between Group A and every other group ($P < 0.01$). The mean values and standard error are indicated by points and bars.

Table 1. Comparison of laboratory findings between uninephrectomized and non-nephrectomized rats receiving aminonucleoside and protamine sulfate[a]

	Uninephrectomized rats Group A, Day			Non-nephrectomized rats Group E, Day		
	0	52	80	0	52	80
Urinary protein[b] *mg/day*	1.6 ± 0.3	574 ± 49	411 ± 70	1.9 ± 0.5	593 ± 32	459 ± 44
Serum albumin[b] *g/liter*	30 ± 1	14 ± 0	23 ± 1	32 ± 1	15 ± 0	18 ± 1
Serum total cholesterol[b] *mmol/liter*	2.3 ± 0.1	18.8 ± 0.3	7.7 ± 0.9	2.1 ± 0.1	15.5 ± 0.7	9.4 ± 1.7
Serum creatinine[c] *μmol/liter*	69 ± 3	218 ± 29	384 ± 58	68 ± 3	64 ± 5	60 ± 2
Serum urea[c] *mmol/liter*	7 ± 1	24 ± 3	70 ± 3	5 ± 0	11 ± 1	11 ± 2
Creatinine clearance[c] *ml/min/kg body wt*	3.0 ± 0.1	1.2 ± 0.2	0.4 ± 0.1	3.5 ± 0.2	3.3 ± 0.4	3.5 ± 0.1

[a] Each value was obtained from 5 rats sacrificed on day 80 and is indicated by mean and standard error
[b] Not significantly different between the groups by analysis of variance
[c] Significantly different between the groups by analysis of variance ($P < 0.01$)

day 24 showed typical focal segmental pattern with hyaline deposits on day 52. Focal tubular atrophy, variable interstitial fibrosis and patchy interstitial, mononuclear cell infiltration were also present (Fig. 4a). On day 80, most of the glomeruli showed advanced changes and widespread interstitial lesions developed (Fig. 4b). The histology was very similar to the end–stage renal disease in human FGS and other forms of sclerosing GN. No distinct change was found in the kidneys of Groups C and D.

Histometrical studies (Table 2). The index of glomerular

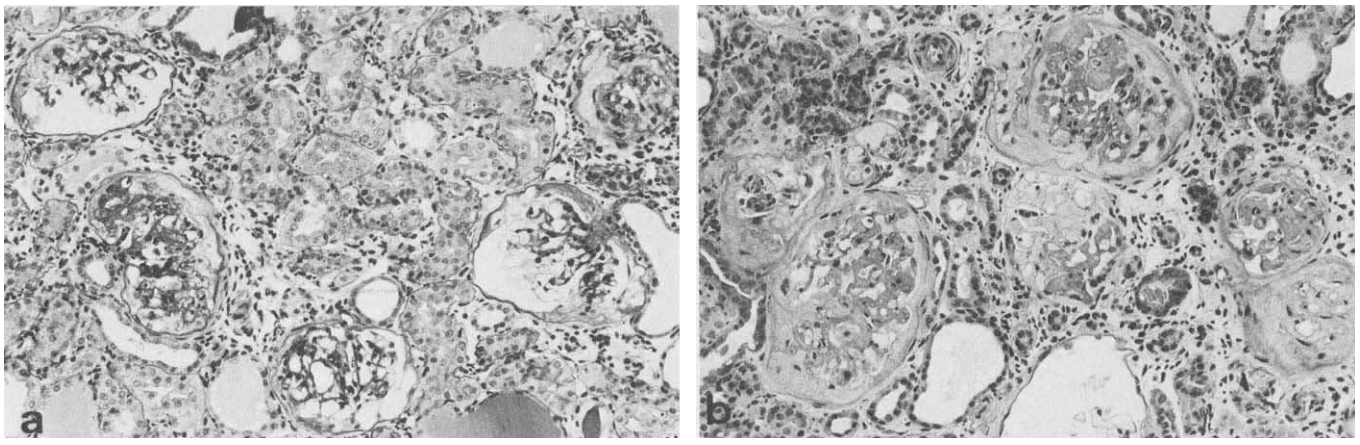

Fig. 4. *Light micrographs of the kidney from rats of Group A on days 52 (a) and 80 (b).* (a) Most of glomeruli show segmental or global sclerosis. Dilatated and atrophied tubuli are seen, but normal tubuli are still present. (b) Glomerular changes are advanced and global sclerosis can be seen. Tubular atrophy and interstitial fibrosis are prominent. (Periodic acid–Schiff, ×100)

Table 2. Index of sclerosis at varying periods in each rat group

Groups[a]	Experimental days of sacrifice	Numbers of rats in group	Index of sclerosis[b]	Percent interstitial volume[b]
Before[c]		5	0.07 ± 0.02	15.2 ± 0.9
A	24	5	0.60 ± 0.08	19.5 ± 1.0
	52	5	1.83 ± 0.11	32.7 ± 2.8
	80	5	2.55 ± 0.06[d]	42.9 ± 2.7[d]
B	24	3	0.26 ± 0.03	17.7 ± 0.3
	52	3	1.58 ± 0.20	25.5 ± 0.5
	80	5	1.65 ± 0.26	30.8 ± 3.5
C	24	3	0.08 ± 0.01	12.2 ± 0.8
	52	3	0.05 ± 0	13.3 ± 1.3
	80	5	0.11 ± 0.03	17.9 ± 0.5
D	80	5	0.09 ± 0.01	11.9 ± 0.9
E	80	5	1.28 ± 0.15	23.6 ± 1.7

[a] Group A, receiving aminonucleoside (AMNS) and protamine sulfate (PS); Group B, AMNS and saline; Group C, PS alone; Group D, saline alone
[b] Means ± SE
[c] Five kidneys randomly obtained from rats at the unilateral nephrectomy 3 weeks before the initial injection
[d] Significantly different, versus each other group on day 80 ($P < 0.01$)

Table 3. Urinary protein and ruthenium red particles on the lamina rara externa in the initial stage

	Day 4		Day 7	
Groups[a]	Urinary protein[b] *mg/day*	Ruthenium red[c] *particles/ μm*	Urinary protein[b] *mg/day*	Ruthenium red[c] *particles/ μm*
A′	7.2 ± 1.4	22.2 ± 0.6[d]	253.3 ± 45.4	20.1 ± 0.5[d]
B′	5.4 ± 0.9	24.4 ± 0.4	27.4 ± 1.8	26.0 ± 0.5
C′	6.0 ± 0.4	26.9 ± 0.7	4.7 ± 1.8	27.9 ± 0.7
Non-treated	2.6 ± 0.2	28.7 ± 0.6		

[a] Group A′, receiving aminonucleoside (AMNS) and protamine sulfate (PS); Group B′, AMNS and saline; Group C′, PS alone
[b] Means ± SE from 3 rats sacrificed
[c] Each value was obtained from 60 sections (20 × 3 rats) of the lamina rara externa and expressed as mean ± SE
[d] Significantly different, versus each other group ($P < 0.01$)

sclerosis was much higher in Group A rats than in rats from Groups B and E. Glomeruli of rats from Groups C and D showed no significant abnormality when compared with the glomeruli in the kidneys removed at uninephrectomy three weeks before the initial injection. The similar results were obtained on the analysis of percent interstitial volumes. Increase of Index of Sclerosis and percent interstitial volume was revealed throughout the course of the experiment when compared between subgroups in Group A. The latter showed that nearly a half was occupied by the interstitial area in the histology of rats in Group A on day 80.

Immunoperoxidase studies. Strongly positive deposits of IgM and moderate deposits of C3 were present in sclerotic areas in glomeruli of Group A rats with advanced lesions (Fig. 5). Weakly positive deposits of IgG and IgA were seen in a segmental distribution only in a few glomeruli. There were similar but less intense deposits of immunoglobulins and C3 in glomeruli of rats from Groups B and E. Apart from a few highlights, no significant immunoglobulin or complement deposition was found in glomeruli of Groups C and D rats.

Studies in the initial stage

Urinary protein excretion rose on day 5 and remained high in Group A′ rats (Table 3). The rats in Group B′ also showed an increase in urinary protein, but less marked.

Light microscopy revealed no distinct change in the histologic appearnce at this stage, although expanded Bowman's spaces and tubular hyaline casts were occasionally seen in the very proteinuric animals. On electron microscopic observation, effacement of foot processes was present along GBM in preproteinuric phase of the AMNS-treated rats on day 4 (Fig. 6). This change was milder in Group B′ rats (Fig. 6b) than in Group A′ rats (Fig. 6a). On day 7, the cytoplasm of the epithelial cells containing many electron dense lysosomes covered the GBM in the glomeruli of Group A′ rats (Fig. 7a), and foot processes completely disappeared. Capillary lumina were occluded by hyaline-like electron dense materials. The similar changes were seen but mild in Group B′ rats (Fig. 7b). Smaller numbers of

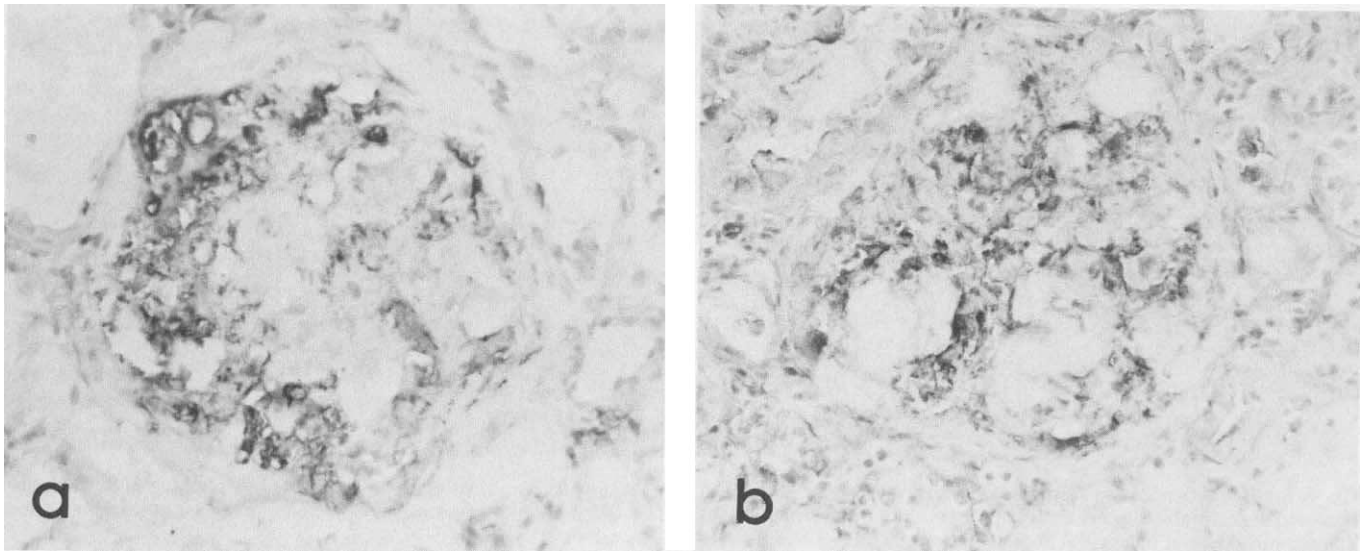

Fig. 5. *The kidney from a rat of Group A on day 80 stained for IgM* **(a)** *and C3* **(b)** *by indirect immunoperoxidase technique.* Heavy deposits of IgM and moderate deposits of C3 are observed in sclerotic area of glomeruli. (×400)

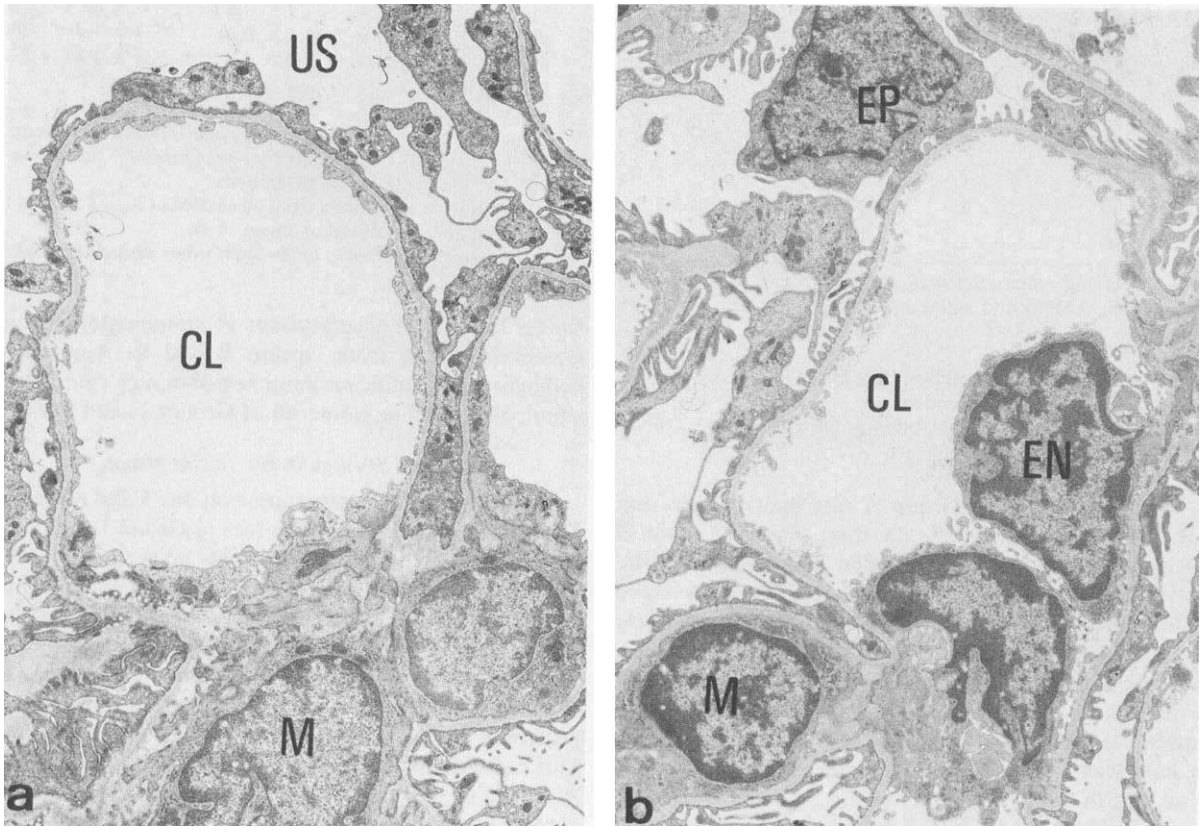

Fig. 6. *Low-power electron micrographs of glomeruli from rats in Group A'(AMNS + PS)* **(a)** *and in Group B'(AMNS + saline)* **(b)** *on day 4.* Effacement of foot processes is more marked in Group A', compared to that in Group B', which show very minor changes. Abbreviations are: US, urinary space; CL, capillary lumen; M, mesangial cell; EN, endothelial cell; EP, epithelial cell. (×5000)

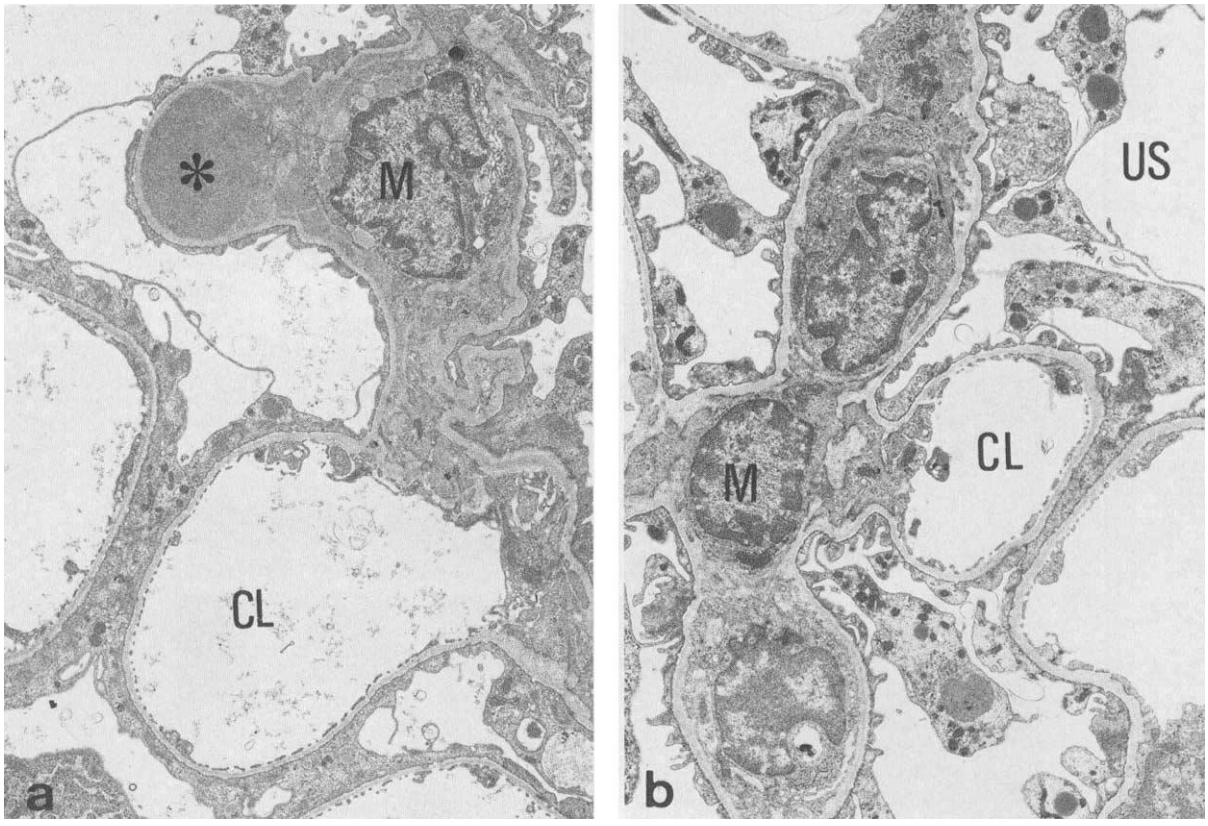

Fig. 7. *Low-power electron micrographs of glomeruli from rats in Group A′ (AMNS + PS)* **(a)** *and in Group B′ (AMNS + saline)* **(b)** *on day 7. A greater degree of epithelial injury is shown in a than in b.* In 7 a there is an extensive effacment of foot processes and an area of bare basement membrane in present on the capillary loop containing a hyaline-like, electron dense lesion (asterisk). Blebs and vacuoles are also present within epithelial cell cytoplasm. These changes were also present in most of group B animals but were much less in degree (b). Abbreviations are: US, urinary space; CL, capillary lumen; M, mesangial cell. (×5000)

particles of RR were deposited on LRE in Group A′ rats than in rats of other groups even in the preproteinuric phase (Fig. 8). The numbers of these particles were significantly different between Group A′ and others on day 4 and 7 respectively (Table 3). The numbers of deposits in Group B′ rats also decreased but were less marked. There was no significant difference between Group C′ and the non-treated rats.

Discussion

In any animal experimental model of human disease it is important not only that the induced lesions closely resemble the lesions in humans, but that the animals live long enough to study the evolution of the disease, the pathogenesis of the lesions, and the effects of therapy. Many different approaches have produced experimental models of FGS, including chemicals [1, 15, 16], aging [17–20], obesity [21], remnant kidneys [22, 23] and protein overloading [24]. However, in these studies the histological changes were restricted mainly to the glomerulus, were usually not sufficiently severe to progress to end-stage renal disease, and thus did not mimic the focal sclerotic lesions found in human FSGS. Our study clearly shows that the combined administration of AMNS and PS in low doses is not lethal to the animals, and is a much more efficient method of inducing FSGS in rats than the administration of AMNS alone. The fact that Group A (AMNS + PS) rats had much more severe sclerotic lesions in their glomeruli than Group B rats (AMNS alone) indicates that glomerular sclerosis in AMNS nephrosis is enhanced by PS. Furthermore, this technique produces an FGS lesion which progresses to end-stage renal failure. This does not occur when AMNS is given by itself.

Many authors [25–28] have shown that AMNS has a toxic effect on the glomerular epithelial cell and induces a loss of charge in the glomerulus which results in proteinuria. Moreover, Velosa et al [2] demonstrated, using colloidal iron staining, that the glomerular sclerotic lesions in chronic AMNS nephrosis were preceded by polyanion loss and irreversible injury to the epithelial cell. In addition it has been demonstrated that PS neutralizes anionic sites in the glomerulus and caused reversible epithelial damage [3–5]. Therefore, the rationale behind this study was to see if PS potentiated the effect AMNS on the glomerular polyanion producing an FSGS which progressed to end-stage renal disease.

The neutralizing effect on glomerular anionic sites by PS has recently been studied in the models of immune complex nephritis, although PS has been assumed to act as an agent for eliminating cationized immune complexes [6]. The effect of PS in the early stages of our experimental model was demonstrated by the ultrastructural findings which showed epithelial lesions and glomerular polyanion loss. RR was used as the marker to indicate negative charges in the glomerulus. Although RR

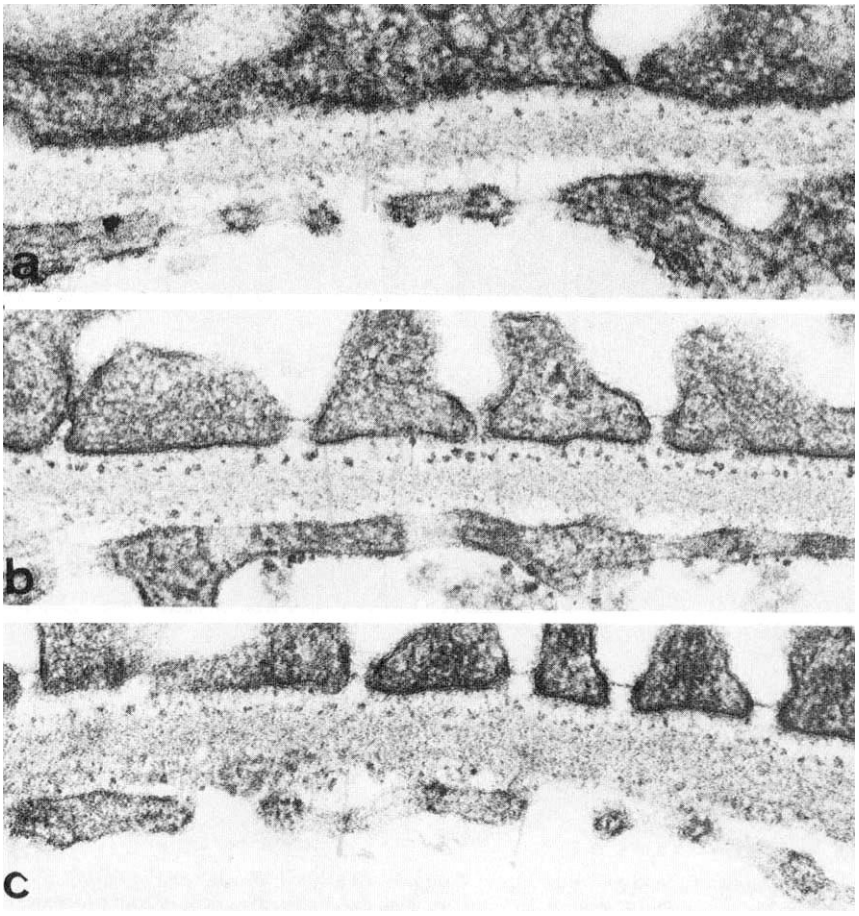

Fig. 8. *Electron micrographs of GBM from rats on day 4 (24-hour after the last administration of drugs) perfused by ruthenium red solution.* **a**. Group A′ (AMNS + PS); **b**, Group B′ (AMNS + saline); **c**, Group C′ (PS alone). RR particles on LRE were markedly diminished in Group A′ rats, compared to rats in Groups B′ and C′. (×78000)

particles are seen in the LRE, the LRI and the cell coats covering the epithelium and the endothelium, it is the easiest to demonstrate the regularity of the particles in the LRE in cross section [13]. In the present experiment RR deposition of the LRE was decreased when PS was administered with AMNS in contrast to that observed by the low dose administration of AMNS alone. This suggests that the mode of action of PS might be to enhance the initial toxic effect of AMNS on the epithelial cell, by accelerating the glomerular polyanion loss and the subsequent excretion of urinary protein and thus inducing glomerular sclerosis as Velosa et al [2] proposed.

In order to minimize the toxicity of PS the dosage and the frequency of the PS-administration was determined from the direct renal perfusion studies of Vehaskari et al [5]. Multiple injections of PS alone neither increased proteinuria nor induced glomerular sclerosis, and the glomerular polyanion loss was not demonstrated. This inability of PS alone at this comparatively low dose to produce glomerular damage has been also demonstrated by other authors [6, 8]. PS is known to have an adverse effect on the systemic circulation when intravenously injected [29–32], and multiple injections of PS in large doses can cause hemodynamic alterations in the kidney and glomerular lesions. However, in our model this mechanism probably does not play a role because of the doses involved, its rapid in vivo inactivation, and because the hemodynamic effect is transient [5].

In our experiments extremely high cholesterolemia was observed. Several authors have reported that PS inhibits lipase activity [33, 34]. Moorhead et al [35] suggested that loss of lipoprotein lipase activators caused by increase GBM permeability leads to abnormal lipid metabolism which could affect mesangial function and have a role in the accumulation of mesangial matrix. This concept was given further support by Grond et al [16, 22] who studied uninephrectomized rats, AMNS nephrosis and adriamycin nephrosis and thought that mesangial dysfunction might be responsible for the eventual glomerular sclerosis. Moreover, studies of obese rats showed that hyperlipidemia was followed by high protein excretion in urine [36] and eventually progressive glomerular sclerotic lesions [21]. Therefore, it may be that the abnormal lipid metabolism is enhanced by PS and may be one of the factors accelerating sclerosis in our FSGS model.

The FSGS induced in unilaterally nephrectomized rats is far more severe than in non-nephrectomized rats. This is in accordance with other studies which have shown that unilateral nephrectomy promotes lesions in the remaining kidney. This effect may be induced by glomerular hyperfiltration postulated by Brenner and Meyer [37] because the reduction in renal mass produced by uninephrectomy results in an increase in the blood

flow, glomerular filtration rate and glomerular capillary pressure in a single nephron.

Spontaneously sclerotic glomerular lesions develop in rats which are more than six months of age [17–20]. It is believed that this sclerosis is either due to an inherited genetic abnormality or the result of repeated exposure of the glomeruli to toxic agents. We have avoided any influence of this spontaneous glomerular sclerosis in our experiments by using young rats and having control groups of the same age.

This experimental model of FSGS with its full spectrum of readily-induced glomerular and interstitial lesions, which closely resemble the changes of progressive human FSGS, should facilitate further study of the pathology and pathogenesis of this condition and the effect of treatment on its relentless course.

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